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# PATENT SPECIFICATION

NO DRAWINGS

**831,124**

*Date of Application and filing Complete Specification May 1, 1956.*

*No. 13406/56.*

*Application made in United States of America on May 18, 1955.*

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Index at acceptance:—**Classes 2(2),** B2C5; **and 2(3),** H2.

International Classification:—**C07g. D01f.**

COMPLETE SPECIFICATION

## Purification of Collagen

We, ALFRED BLOCH, of 515 So. Fourth Avenue, Highland Park, New Jersey (Middlesex County), United States of America, and IRVING BERNT ONESON, of 40 Brookside Avenue, Somerville, New Jersey (Somerset County), United States of America, both Citizens of the United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and

heretofore used have been characterised by marked degradation of the collagen molecules and a subsequent decrease in tensile strength. The decrease in tensile strength of strands of collagen prepared according to methods heretofore used has been of such an order that they were not acceptable to the surgical profession for use in suturing and ligating. The reason for the loss of tensile strength is considered to

SPECIFICATION NO. 831,124

By a direction given under Section 17(1) of the Patents Act 1949 this application proceeded in the name of Ethicon, Inc. a Corporation organised under the laws of the State of New Jersey, United States of America, of U.S. Highway 22, Bridgewater Township, New Jersey, United States of America.

THE PATENT OFFICE,
*16th August, 1960*

DS 79719/1(13)/4023 200 8/60 DL

preparation of collagen articles in the form of films, tubes, casings, sponges, and the like, and particularly for the preparation of collagen strands for use in suturing and ligating. Heretofore, crude collagenous materials were treated by physical means to remove extraneous matter including fat and muscle tissue and an aqueous dispersion of the collagen was then prepared. In preparing the dispersion, the collagen was swollen in water, dilute acid or alkali, macerated by physical means and then dispersed in a dilute aqueous acid solution. Non-dispersible matter was removed from the dispersion of collagen and the purified dispersion was treated in a variety of ways to produce the desired shaped collagen article. Collagen strands have been produced by extruding the dispersion in a continuous manner into a liquid such as acetone which is capable of precipitating the collagen in the dispersion. Shaped collagen articles prepared by methods

to form the dispersions. An increase in the time during which the collogen is dispersed in a suitable medium also results in degradation of the native collagen.

The basis of the resent invention is the discovery that collagenous materials may be purified by an enzymatic digestion, and subsequent removal, of the non-collagenous materials and that the purified collagen may be readily dispersed in a suitable medium and preferably an aqueous medium. Regenerated collagen and particularly strands of regenerated collagen which have markedly improved tensile strength may be obtained by precipitation of the collagen in the dispersion. Native collagen from which fat, muscle or other extraneous matter has been physically removed is sliced or reduced to particles of small size by any appropriate mechanical means and then treated with an enzyme solution which is inert to collagen but capable of acting on and dissolving non-

[*Price 3s. 6d.*]

Price 4s 6d

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This invention relates to the purification of collagenous material and more particularly relates to the removal of non-collagenous substances from collagenous material by enzymatic means, whereby purified collagenous material is produced which may be rapidly and substantially completely dispersed in a medium with a low degree of degradation of the collagen.

Numerous attempts over many years have been made to utilize mammalian collagenous tissue such as tendons and bladders, as well as fish bladders, as a source of collagen for the preparation of collagen articles in the form of films, tubes, casings, sponges, and the like, and particularly for the preparation of collagen strands for use in suturing and ligating. Heretofore, crude collagenous materials were treated by physical means to remove extraneous matter including fat and muscle tissue and an aqueous dispersion of the collagen was then prepared. In preparing the dispersion, the collagen was swollen in water, dilute acid or alkali, macerated by physical means and then dispersed in a dilute aqueous acid solution. Non-dispersible matter was removed from the dispersion of collagen and the purified dispersion was treated in a variety of ways to produce the desired shaped collagen article. Collagen strands have been produced by extruding the dispersion in a continuous manner into a liquid such as acetone which is capable of precipitating the collagen in the dispersion. Shaped collagen articles prepared by methods heretofore used have been characterised by marked degradation of the collagen molecules and a subsequent decrease in tensile strength. The decrease in tensile strength of strands of collagen prepared according to methods heretofore used has been of such an order that they were not acceptable to the surgical profession for use in suturing and ligating. The reason for the loss of tensile strength is considered to be that the collagen molecules or aggregates of collagen molecules present in native collagen have been degraded during the preparation of the dispersion. Attempts have been made to minimize degradation of native collagen during the preparation of collagen dispersions by using only small amounts of acid or alkali in preparing the collagen dispersion and also by preparing the dispersions at a low temperature; however, such expedients have not resulted in the production of shaped collagen articles such as strands of collagen having substantially improved tensile strength because the use of weaker acids or a lower concentration of acid and the preparation of the dispersion at a low temperature have increased the time required to form the dispersions. An increase in the time during which the collogen is dispersed in a suitable medium also results in degradation of the native collagen.

The basis of the resent invention is the discovery that collagenous materials may be purified by an enzymatic digestion, and subsequent removal, of the non-collagenous materials and that the purified collagen may be readily dispersed in a suitable medium and preferably an aqueous medium. Regenerated collagen and particularly strands of regenerated collagen which have markedly improved tensile strength may be obtained by precipitation of the collagen in the dispersion. Native collagen from which fat, muscle or other extraneous matter has been physically removed is sliced or reduced to particles of small size by any appropriate mechanical means and then treated with an enzyme solution which is inert to collagen but capable of acting on and dissolving non-

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collagenous components such as, for example, elastin, mucopolysaccharides. Crude malt diastase derived from barley as well as enzymes derived from animal pancreatic tissue and from microorganisms, particularly of the B. subtilis group, have been found capable of removing noncollagenous components from native collagen without having any degrading effect on the collagen itself.

The invention is best practised as follows:—

Finely divided native collagen, substantially free from fat and muscle tissue, is treated with a solution of the enzyme at a temperature within the range of from 5° C. to 45° C. for a time sufficient to remove substantially completely noncollagenous components. At a temperature below about 5° C. the reaction is extremely slow and at a temperature above 45° C. the enzyme is substantially inactivated. The minimum time required for the treatment is about ten hours and is related to the temperature of the treating solution, the time of the treatment being shorter at higher temperatures. When the time of treatment is shorter than about ten hours at a temperature low in the above range the noncollagenous components are incompletely removed. The pH of the treating solution is adjusted to a point substantially within the range of from pH 5 to pH 9 and preferably from pH 8.0 to 9.0. At a pH substantially at the iso-electric point, swelling and degradation of collagen is at a minimum.

After treatment with an enzyme solution, the separately finely divided collagen is dispersed in a suitable medium and preferably an aqueous medium. The time required for the formation of the collagen dispersion is markedly reduced by the pretreatment of the finely divided native collagen with the enzyme solution. Unswollen particles are removed from the collagen dispersion by decantation, filtration, and preferably by centrifugation, and shaped collagen articles, and particularly collagen strands, are prepared by precipitation of regenerated collagen from the collagen dispersion. The shaped articles of regenerated collagen so prepared, and in particular collagen strands, have markedly increased tensile strengths.

The following examples are given to illustrate the process of this invention:—

### Example I

Fresh bovine Achilles' tendons from which the fat and fascia had been mechanically removed were sliced across the grain to provide slices 1 to 2 mm. thick. 20 grams of the tendon slices were added to 200 cc. of an enzyme solution containing one per cent by weight of malt diastase, USP IX, buffered at a pH of 8. The enzyme solution containing the tendon silces was incubated for sixteen hours at a temperature of 37° C. The tendon slices were then removed from the enzyme solution by filtration, washed with distilled water, and added to 400 cc. of a 0.3 per cent aqueous solution of acetic acid and allowed to remain in the acetic acid solution for 20 hours at room temperature. The acetic acid dispersion was centrifuged and the supernatant liquid was removed by decantation. 70 cc. of supernatant liquid were obtained. The supernatant liquid was added to the swollen slices from which it had been previously decanted and the volume of the mixture was increased to two litres by the addition of distilled water. The mixture was mechanically dispersed in a Waring blender and then filtered through the open holes of a Buchner funnel to remove large unswollen collagen particles. The filtrate was diluted to a volume of four litres with distilled water and centrifuged. The clear effluent obtained by decantation following centrifugation was neutralised with ammonia and this resulted in precipitation of the collagen in fibrous form. The precipitated collagen was removed from the solution and dried and the weight of the dried collagen was five grams. The above procedure was repeated using the same amount of bovine tendon collagen except that the enzyme treatment was omitted, and only two grams of dried precipitated collagen were obtained. The supernatant liquid had a volume of 265 cc.

### Example II

10 grams of bovine Achilles' tendon slices, prepared as in Example I, were added to 100 cc. of an enzyme solution containing 1 per cent by weight of malt diastase, US PIX, buffered at a pH of 8.5. The mixture was incubated at 37° C. for sixteen hours. The tendon slices were removed from the mixture, washed with distilled water, and sufficient 0.3 per cent aqueous acetic acid solution was added to the washed slices to bring the volume of the mixture to 200 cc. The dispersion in aqueous acetic acid was allowed to stand at room temperature for twenty hours and then centrifuged. 52 cubic centimetres of supernatant liquid were obtained upon decantation of the centrifuged dispersion.

The procedure above was repeated using 10 grams of bovine Achilles' tendon slices and an enzyme obtained from an organism of the B subtilis type (HT concentrate, obtained from Takamine Laboratories, Inc., Clifton, N.J.), in place of malt diastase, and in this instance, 36 cc. of supernatant were obtained. In order to show the effect of the enzyme treatment in the volume of collagen dispersion obtained, the above procedure was repeated with the omission of the treatment of finely divided collagen with the enzyme solution, and in this instance, 134 cc. of supernatant liquid were obtained following centrifugation.

### Example III

Fresh beef hide, from which hair and muscle

tissue had been removed, was cut into narrow strips and passed through a meat grinder. 30 grams of ground hide were added to 100 cc. of a filtered two per cent dispersion of malt diastase, USP IX, and the resulting mixture was incubated for sixteen hours at 37° C. The enzyme solution was decanted and the finely divided beef hide was washed with distilled water and added to 600 cc. of a five per cent aqueous solution of acetic acid. The mixture was allowed to stand at room temperature for 48 hours and then filtered through cheesecloth. 94 cubic centimetres of filtrate were obtained. The above procedure was repeated but in this instance, the finely divided beef hide was not subjected to the action of the enzyme solution and 115 cc. of filtrate were obtained. The filtrates in both instances were recombined with the finely divided beef hide, diluted with three litres of distilled water and dispersed in a Waring blender. The dispersions were filtered through the open holes of a Buchner funnel to remove large unswollen pieces. The filtrates were each diluted with two litres of distilled water and centrifuged. The clear effluents were free from unswollen particles and were neutralized with ammonia. The precipitated collagen fibres in each instance were removed, washed with acetone, and dried. Four grams of dried collagen fibres were obtained from the process in which the finely divided beef hide was subjected to the enzyme treatment whereas only 1.3 grams of dried collagen fibres were obtained from the process in which the enzyme treatment was omitted.

### Example IV

Collagen from tendons, purified according to the method of Example I, was used in the preparation of a collagen gel. Five grams of dry precipitated collagen in fibrous form were swollen in sufficient 0.3 per cent by weight aqueous malonic acid solution to provide a gel having a collagen content of 1.6 to 1.8 per cent by weight. The gel was homogenised in a Waring blender and then centrifuged at 30° C. to remove air bubbles. The collagen gel was extruded under a constant pressure of twenty pounds of nitrogen as a multifilament into a coagulating bath of circulating acetone containing sufficient ammonia to neutralise the malonic acid in the gel. The spinnerette through which the collagen gel was extruded consisted of 40 elogated tubes having an internal diameter of 15 mils, the 40 tubes being arranged in two concentric rows. The speed at which the collagen gel was extruded varied from 0.03 to 0.06 cc. of gel per second and the spinnerette was rotate at a speed of 0.04 to 1.0 revolution per second. The extruded strand was removed from the coagulating bath on a godet which imposed a stretch of about 500 per cent to the strand. The stretched strand was air-dried and subsequently stretched an additional 30 per cent. The above procedure was repeated an additional two times and the three stretched strands so obtained had diameters of 10.5, 9.5, and 7.5 mils, tensile strengths of 5.88, 6.0, and 5.0 pounds, and strengths measured in grams per denier of 2.66, 2.47, and 3.80, respectively.

A further collagen strand produced in a manner similar to the above method except that the step in the preparation of the collagen gel of treatment with an enzyme was not included, had a diameter of 10.5 mils, a tensile strength of 4.0 pounds, and a strength measured in grams per denier of 2.22 grams.

WHAT WE CLAIM IS: —

1. Process for preparing a dispersion of collagen in a suitable dispersion medium, in which as an initial step finely divided native collagen is purified by removal of non-collagenous material by enzymatic digestion.

2. Process according to Claim 1 in which the enzymatic digestion is performed with a solution of an enzyme capable of acting on and dissolving noncollagenous substances for a period of at least about ten hours at a temperature of from 5° C. to 45° C., the pH of the treating solution being from five to nine.

3. Process according to Claim 2 in which the treating solution has a pH of from eight to nine.

4. A process of preparing a dispersion of collagen for the production of shaped articles comprising the following steps: physically removing extraneous matter from mammalian collagenous tissue; subdividing the collagenous tissue; treating the latter with a solution of an enzyme capable of acting on and dissolving noncollagenous substances for a period of at least about ten hours at a temperature of from 5° C. to 45° C., the pH of the treating solution being from five to nine; and dispersing the treated material in a dispersive medium.

5. A process according to Claim 4 in which the pH of the treating solution is from eight to nine.

6. Process according to Claim 4 or 5 in which the medium is an aqueous acid.

7. Process according to Claim 6 in which the acid is acetic acid or malonic acid.

8. Process according to Claim 6 or 7 in which the collagen is precipitated in shaped form in a liquid comprising an agent to neutralise the acid.

9. Process according to any of the preceding claims in which the collagen is obtained from any fresh tendons or hides.

10. Process according to any of the preceding claims in which the enzyme is malt diastase, or pancreatin, or an organism of the B. subtilis type.

11. Process for preparing collagen substantially as described in any of the foregoing examples.

CARPMAELS & RANSFORD,
Agents for Applicants,
24, Southampton Buildings, Chancery Lane,
London, W.C.2.

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